Impact of cleaning before obtaining midstream urine samples from children

Rebekka Lytzen[1], Jenny Dahl Knudsen[2], Steen Ladelund[3], Marianne Soendergaard Khinchi[1] & Dina Cortes[1, 4]

ABSTRACT

INTRODUCTION: Microbiological documentation of one uropathogenic bacterium in significant numbers in urine from patients with typical symptoms is the gold standard for diagnosing urinary tract infection (UTI). Cleaning before collecting midstream urine (MSU) is reported not to reduce the risk of contaminating the sample and was therefore omitted at Hvidovre Hospital as from the autumn of 2006. We evaluate if no cleaning increased the risk of contamination in the Department of Paediatrics.

MATERIAL AND METHODS: A total of 1,858 patients aged 0-15 years who were suspected of UTI delivered two MSUs within 24 h. In 2004-2006 ("cleaning period"), 523 children were cleaned before obtaining two MSUs, contrary to the 1,335 children included in 2008-2010 ("non-cleaning period"). Significant bacteriuria was defined as at least 10,000 colony-forming units/ml of the same uropathogenic bacterium in two MSUs in monoculture. Contamination was defined as all other microbiological findings.

RESULTS: The procedure of no cleaning before sampling increased the risk of contamination in 0-9.9-year-old children from 43% to 49% (p = 0.034); and specifically in 0-9.9-year-old girls, the risk of contamination increased from 47% to 55% (p = 0.018). No significant effect was demonstrated in 10-15-year-old girls (p = 1.0) or in boys, independent of age (p = 0.19). In both periods, 31% of paired MSUs from the same child were without any bacterial or fungal growth.

CONCLUSION: Cleaning before collecting urine from girls younger than ten years of age is recommended to minimise the risk of contamination. Cleaning was without effect on children aged 10-15 years.

FUNDING: not relevant.

TRIAL REGISTRATION: not relevant.

Urinary tract infections (UTIs) commonly occur in childhood. Thus, 8% of girls and 2% of boys have been diagnosed with at least one UTI at the age of seven [1, 2]. Microbiological examination of a urine sample is mandatory for diagnosing UTI in children as well as in adults.

It is important to avoid contamination of the urine samples since this can lead to a false diagnosis of UTI, which may imply unnecessary antimicrobial treatment and urological investigations [1-3].

In adults, several studies have reported no impact of genital and perineal cleaning before obtaining midstream urine (MSU) [4-10]. Similarly, in toilet-trained children older than two years of age, cleaning did not reduce the risk of contamination of MSUs [11-13]. Consequently, from the autumn 2006 routine cleaning before obtaining MSUs was stopped at Hvidovre Hospital, except in soiled patients.

We focused on contamination and evaluated if the procedure of no routine cleaning before providing MSUs had increased the risk of contamination in 0-15-year-old children suspected of UTI. To our knowledge, our study is the first to include children who have not been toilet-trained.

MATERIAL AND METHODS

Patients and settings

Included were consecutive patients 0-15 years of age, admitted to the Department of Paediatrics, Hvidovre Hospital, Denmark, from 1 January 2004 to 31 December 2010 on suspicion of UTI, who provided two MSUs within 24 h. Patients were only included once, in connection with their first possible episode. The urine samples were initially examined for leukocyte esterase or nitrites by dipstick. If this first voided MSU was dipstick-positive or there was a strong clinical suspicion of UTI, the urine was sent for microbiological examination, as was a second MSU sample taken within 24 h after the first.

This evaluation was performed as a before-and-after study using the two period+s "cleaning period", from 1 January 2004 through 30 June 2006; and "non-cleaning period", from 1 January 2008 through 31 December 2010, when no cleaning was performed before sampling, unless the patients were soiled. In both periods, soiled patients were cleaned with soap and water.

In the cleaning period, the children were routinely cleaned with sterile swabs moistened in 0.9% saline before obtaining the two MSUs. Cleaning was carried out by nurses in The Department of Paediatrics.

Apart from the systematic cleaning in the cleaning period, the procedures in the two periods were identical. If sampling was done by the patients themselves or by their parents, they were instructed and informed not to rub the container on the perineal skin or to handle

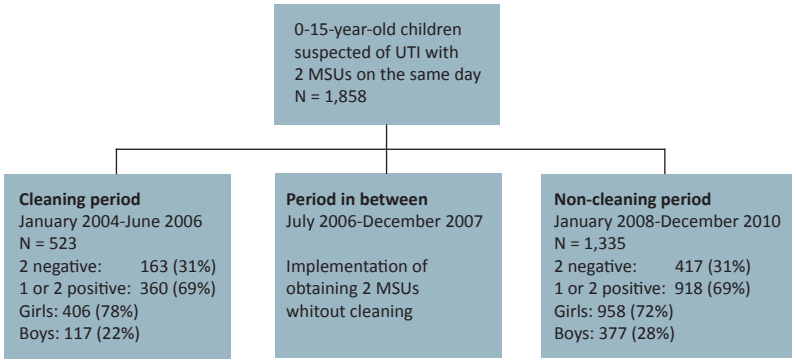
ORIGINAL ARTICLE

1) Department of Paediatrics, Hvidovre Hospital
2) Department of Clinical Microbiology, Hvidovre Hospital
3) Clinical Research Centre, Hvidovre Hospital
4) Faculty of Health Science, University of Copenhagen

Dan Med J 2014;61(6):A4861

FIGURE 1

The study design and the number of patients included.



MSU = midstream urine sample; UTI = urinary tract infection.

the inside of the container. The majority of the boys were not circumcised.

The MSU samples were transferred to a sterile container and kept at 4 °C until transportation to the Department of Clinical Microbiology, which was done on a daily basis. Nothing was added to the urine samples.

The Department of Paediatrics is a secondary referral paediatric centre. In the 2004-2010 period, the catchment area increased by about 40%: from 87,900 to 121,600 children aged 0-15 years (Statistics Denmark [14]).

Data source

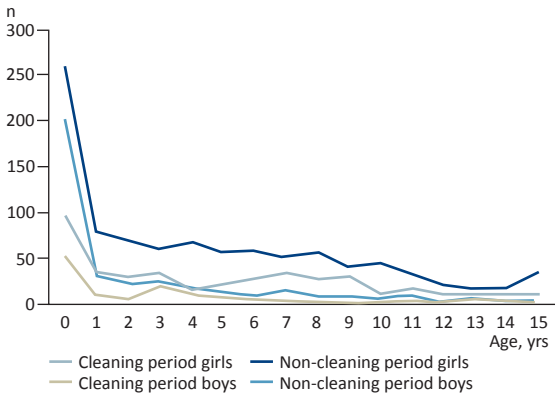
From the laboratory database at the Department of Clinical Microbiology, Hvidovre Hospital, data on sex, age, date of sampling and the results of the microbiological examination (i.e. identification and quantification of bacteria and fungi, and the susceptibility patterns obtained) were retrieved using the unique identification number of all Danish citizens.

Excluded were patients admitted from 1 July 2006 to 31 December 2007 as the procedure of no cleaning was introduced in autumn 2006 and not consistently implemented until 1 January 2008.

Patients with urine samples obtained through suprapubic aspiration were not included.

FIGURE 2

The age and sex of 1,858 patients suspected of urinary tract infection, and from whom two midstream urine samples within 24 h were examined in the two time periods with cleaning, and without cleaning before sampling.



Urine examination and urinary tract infection classification

Urine samples were analysed on a daily basis. The examination of urinary samples included the inoculation of 10 µl of urine on each of three agar media and incubation for approximately 20 h in ambient air at 35 °C, followed by species identification and susceptibility tests according to the routine procedures at the Department of Clinical Microbiology.

UTI was defined when both MSU samples showed significant growth (> 10,000 colony-forming units (cfu)/ml of urine) of the same uropathogen, and the patient had clinical signs and symptoms of UTI [15].

Species regarded as uropathogens included *Escherichia coli, Klebsiella* species, other *Enterobacteriacea, Enterococcus* species, *Staphylococcus saprophyticus*, haemolytic streptococci, *Aerococcus* species, and *Pseudomonas aeruginosa.*

Contamination was defined as any other findings than monoculture of the same uropathogen in the two MSUs [11-13, 16]. Therefore, the patients with contamination could have UTI *and* contamination or just contamination. The patients with contamination were further classified as follows: a) those with presence of two or more different bacteria, polybacteria. This was defined as those with more than one uropathogen, those with skin and genital flora (coagulase-negative staphylococci (except for *S. saprophyticus*), Lactobacillus species and *Corynebacterium* species) and those with a mixed flora; b) those with an insignificant number of a uropathogen, i.e. less than 10,000 cfu/ml in monoculture in at least one of the two MSUs; and c) those with a uropathogenic bacterial species in significant numbers in only one of the two MSUs.

If there were no microbiological findings in either of the MSUs, the patients were designated as “patients with negative samples”.

Ethics

The study was conducted according to the Helsinki II Declaration and accepted by the Danish Data Protection Agency (Journal no. 2010-41-4430).

Statistical analyses

Categorical variables were tested with χ^2 or with Fisher's exact test when the number of patients in one group was below 40. Furthermore, Wilcoxon's rank sum test was used. The analyses were done two-tailed. The level of significance was set to 0.05, and all analyses were performed using SPSS version 19 (IBM, Chicago, USA).

Trial registration: not relevant.

RESULTS

In total, 1,858 patients were included in the two periods, 523 in the cleaning period and 1,335 in the non-cleaning period, respectively (**Figure 1**). In both periods, most of the patients were girls, 78% in the cleaning period and 69% in the non-cleaning period. In both periods, 31% of the patients had negative samples.

The distribution of children according to age is shown in **Figure 2**. The age distribution was identical in the two periods, i.e. the frequency of the 0-9.9-year-old children was 86% both in the cleaning and in the non-cleaning period (p = 1.0). The median age of the patients was 3.8 years in the cleaning period versus 3.1 in the non-cleaning period (p = 0.044).

In children aged 0-9.9 years, omitting cleaning before collecting MSUs increased the risk of contamination from 43% to 50% (p = 0.0252), whereas cleaning was without effect for the 10-15-year-old patients (p = 1.00), **Table 1**.

Specifically, the risk of contamination increased significantly in girls when cleaning was omitted. When stratified by age, this increase was significant in the 0-9.9-year-old girls (p = 0.0184), but not in the 10-15-year-old girls without cleaning, (Table 1). The numbers of boys were smaller, and the risk of contamination when omitting cleaning did not reach the level of significance, irrespective of the boys' age, Table 1.

Girls consistently had a higher risk of contamination than boys: 47% versus 30% in the cleaning period (p = 0.0010) and 54% versus 37% in the non-cleaning period (p = 0.0057).

The types of contamination found in the MSUs examined in the two periods are shown in **Table 2**. Omitting cleaning significantly increased the risk of polybacteria in 0-9.9-year-old patients (p = 0.0235), but no other significant changes in the characteristics of contamination occurred when the procedure was changed.

The frequency of significant growth (> 10.000 cfu/ml of urine) of the same uropathogen in the 0-9.9-year-olds decreased from 28% (124/448) to 21% (235/1143) (p = 0.00013) when cleaning was omitted. *E. coli* was the most frequent uropathogen, found in 85% (339/397) of cases with significant bacteriuria. The frequency of patients with two negative MSUs was constant, 31%

TABLE 1

Risk of contamination in one or two midstream urine samples in regards to sex, age and cleaning regiment in 1,858 patients suspected for urinary tract infection. The values are n (%).

	Cleaning	No cleaning	p-value
Girls			
0-9.9 yrs	162 (47)	436 (55)	0.0184[a]
10-15 yrs	30 (48)	78 (47)	1.0[b]
Total	192 (47)	514 (54)	0.0365[a]
Boys			
0-9.9 yrs	32 (31)	132 (38)	0.2225[b]
10-15 yrs	3 (25)	8 (31)	1.0[b]
Total	35 (30)	140 (37)	0.1882[b]
All patients			
0-9.9 yrs	194 (43)	568 (50)	0.0252[a]
10-15 yrs	33 (44)	86 (45)	1.0[b]
Total	227 (43)	654 (49)	0.0341[a]

a) Pearson's χ^2-test
b) Fisher's exact test

TABLE 2

The types of contamination in 1,858 patients suspected for urinary tract infection who delived two midstream urine samples, classified into those who underwent cleaning and those without cleaning before delivering two midstream urine samples. The values are n (%).

	Cleaning	No cleaning	p-value
Polybacterial			
> 1 typical uropathogen:	30 (7)	133 (12)	0.0032[b]
0-9.9 yrs	2 (3)	12 (6)	0.3619[b]
10-15 yrs			
Total	32 (6)	145 (11)	0.0015[b]
Skin and genital flora:			
0-9.9 yrs	33 (7)	62 (5)	0.1578[b]
10-15 yrs	9 (12)	15 (8)	0.3406[b]
Total	42 (8)	77 (6)	0.0918[a]
Other polybacterial findings:			
0-9.9 yrs	14 (3)	62 (5)	0.0663[b]
10-15 yrs	9 (12)	20 (10)	0.6687[b]
Total	23 (4)	82 (6)	0.1795[b]
All:			
0-9.9 yrs	77 (17)	257 (22)	0.0235[a]
10-15 yrs	20 (27)	47 (24)	0.7541[b]
Total	97 (19)	304 (23)	0.0539[a]
Pure culture of a typical uropathogen in a concentration below 10,000 cfu/ml			
0-9.9 yrs	38 (8)	117 (10)	0.3029[b]
10-15 yrs	2 (3)	9 (5)	0.7331[b]
Total	40 (8)	126 (9)	0.2406[b]
1 MSU with bacteria and one MSU without bacteria			
0-9.9 yrs	79 (18)	194 (17)	0.8097[a]
10-15 yrs	11 (15)	30 (16)	1.0[b]
Total	90 (17)	224 (17)	0.8795[a]

cfu = colony-forming units; MSU = midstream urine sample.
a) Pearson's χ^2-test
b) Fisher's exact test

(163/523) in the cleaning period and 31% (417/1335) in the non-cleaning period, (p = 1.00), Figure 1.

DISCUSSION

We found that no cleaning before obtaining two MSUs increased the risk of contamination in 0-9.9-year-old children, especially in girls. In contrast, we found no effect of cleaning in children aged 10-15 years, or in boys irrespective of age, However, the number of included patients aged 10-15 years and the number of boys were not that high

Our results are not in full agreement with the previously published data on 2-14-year-old healthy toilet-trained children, in whom cleaning before obtaining one MSU was without effect [11-13]. These studies were based on smaller numbers of healthy children, 102 [11, 12] and 99 patients [13], respectively, and the risk of contamination increased without cleaning, although the increase was insignificant. However, Vaillancourt et al demonstrated that cleaning reduces the risk of contamination in MSUs in 2-18-year-old toilet-trained sick children [16]. Our finding that cleaning had no effect on contamination rates in children above ten years of age is in line with the current literature on adults [4-9, 17].

The number of included patients was about twice as high per year in the non-cleaning period as in the cleaning period. The catchment area of our hospital increased from 2004 to 2010, which could explain about 40% of this increase, and we can speculate on the reasons for the remaining increase in number of patients. It has been reported that when cleaning was omitted in children, it increased the risk of positive findings on a dipstick for leukocyte esterase or nitrite from MSUs by a factor 1.8, which subsequently lead to 1.8 times more microbiological analyses [16]. This could be an explanation for the increase in the microbiological examination rate. However, the inclusion criteria of two MSU analysed at the Department of Microbiology remained constant throughout both periods, and the frequency of patients with negative samples was similar, i.e. 31%, in both periods.

At the Department of Paediatrics, Hvidovre Hospital, it is standard practice to obtain two MSUs from children suspected for UTI. This enabled us to find twice as high a risk of contamination as in studies where only one MSU was obtained [11-13, 16]. We found that 17% of the included patients had one MSU with bacteria and one without growth. With only one MSU analysed, not all of these cases would have been characterised as contamination. Furthermore, at the Department of Microbiology, Hvidovre Hospital, 3 × 10 µl of urine is analysed from a MSU from children. This is equivalent to the amount of urine analysed from a suprapubic puncture and ten times as much as the 1 µl inoculation volume obtained in previous studies [11, 12], which can explain the high frequency of contamination in the present study.

Despite cleaning, the risk of contamination in two MSUs was 43% in 0-9.9-year-old children. Collecting MSUs without contamination is difficult, especially in infants and young children [18]. Infant girls are often lying down when they deliver MSUs, and the urine may touch the skin before it is collected in the container. With a risk of contamination reaching the level reported here, it is important to find ways of collecting urine without contamination. In children younger than two years of age, a suprapubic aspiration is an effective way of collecting urine. It is recommended as the gold standard for obtaining urine when UTI is suspected in children younger than two years of age [1-3, 19]. The technique has very limited risks, but technical expertise and experience are required.

Therefore, we recommend suprapubic aspiration in 0-1-year-old patients to reduce the risk of contaminated urine samples. In toilet-trained girls, it is indicated to instruct the girls to sit in reverse on the toilet seat, or to squat, so that the urine does not touch the skin before it is collected as a MSU [12]. Furthermore, boys who are not circumcised have to retract the foreskin as much as possible without pain to reduce the contact between the urine and the skin [1, 2, 13, 20].

In conclusion, we found that cleaning before obtaining MSUs significantly decreases the risk of contamination in girls younger than ten years of age, and cleaning is therefore recommended. We have changed our procedures at the Department of Paediatrics. Despite cleaning, the risk of contamination was high, and to diminish contamination we recommend suprapubic aspiration of urine in 0-1-year-old children, reverse seating on the toilet seat for toilet trained girls and retraction of the foreskin for boys. In contrast, cleaning did not decrease the risk of contamination in 10-15-year-old children and is therefore not recommended in these patients. The numbers, however, were small in this group, so further studies are indicated.

CORRESPONDENCE: *Dina Cortes*, Børneafdelingen, Hvidovre Hospital, Kettegård Allé 30, 2650 Hvidovre, Denmark. E-mail: dina.cortes@regionh.dk.

ACCEPTED: 10 April 2014

CONFLICTS OF INTEREST: none. Disclosure forms provided by the authors are available with the full text of this article at www.danmedj.dk.

ACKNOWLEDGEMENTS: We would like to extend our gratitude to *Niels Frimodt-Møller* for review of the manuscript.

LITERATURE

1. American Academy of Pediatrics. Committee on Quality Improvement. Subcommittee on Urinary Tract Infection. Practice parameter: the diagnosis, treatment, and evaluation of the initial urinary tract infection in febrile infants and young children. Pediatrics 1999;103:843-52.
2. Hansen A, Andersen KV, Cortes D et al. A reference program for children with urinary tract infection. A proposal for diagnosis and treatment of children with urinary tract infection. Ugeskr Læger 1999;161:5775-7.
3. American Academy of Pediatrics. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Clinical

Guideline. Urinary tract infection: Clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. Pediatrics 2011;128:595-610.
4. Lipsky BA, Inui TS, Plorde JJ et al. Is the Clean-Catch midstream void procedure necessary for obtaining urine culture specimens from men? Am J Med 1984;76:257-62.
5. Baerheim A, Digranes A, Hunskaar S. Evaluation of urine sampling technique: bacterial contamination of samples from women students. Br J Gen Pract 1992;42:241-3.
6. Immergut MA, Gilbert EC, Frensilli FJ et al. The myth of the clean catch urine specimen. Urol 1981;27:339-40.
7. Holliday G, Strike PW, Masterton RG. Perineal cleansing and midstream urine specimens in ambulatory women. J Hosp Infect 1991;18:71-5.
8. Leisure MK, Dudley SM, Donowitz LG. Does a clean-catch urine sample reduce bacterial contamination? N Engl J Med 1993;328:289-90.
9. Blake DR, Doherty LF. Effect of perineal cleansing on contamination rate of mid-stream urine culture. J Pediatr Adolesc Gynecol 2006;19:31-4.
10. Lifshitz E, Kramer L. Outpatient urine culture: does collection technique matter? Arch Intern Med 2000;160:2537-40.
11. Lohr JA, Donowitz LG, Dudley SM. Bacterial contamination rates for non-clean-catch and clean-catch midstream urine collection in boys. J Pediatr 1986;109:659-60.
12. Lohr JA, Donowitz LG, Dudley SM. Bacterial contamination in voided urine collections in girls. J Pediatr 1989;114:91-3.
13. Saez-Llorens X, Umana MA, Odio CM et al. Bacterial contamination rates for non-clean-catch and clean-catch midstream urine collection in uncircumcised boys. J Pediatr 1989;114:93-5.
14. Statistics Denmark. www.statistikbanken.dk/BEF1A, www.statistikbanken.dk/BEF1A07, www.statistikbanken.dk/statbank5a/SelectVarVal/Define.asp?MainTable=FOLK1&PLanguage=0&PXSId=0 (13 July 2011).
15. Aspevall O, Hallander H, Gant V et al. European guidelines for urinalysis: a collaborative document produced by European clinical microbiologists and clinical chemists under ECLM in collaboration with ESCMID. Clin Microbiol Infect 2001;7:173-8.
16. Vaillancourt S, McGillivray D, Zhang X et al. To clean or not to clean: Effect on contamination rates in midstream urine collections in toilet-trained children. Pediatrics 2007;119:e1288-e1293.
17. Schneeberger C, van den Heuvel ER, Erwich JJ et al. Contamination rates of three urine-sampling methods to assess bacteriuria in pregnant women. Obstet Gynecol 2013;121:299-305.
18. Alam MT, Coulter JB, Pacheco J et al. Comparison of urine contamination rates using three different methods of collection: clean-catch, cotton wool pad and urine bag. Ann Trop Paediatr 2005;25:29-34.
19. Long E, Vince J. Evidence behind the WHO guidelines: Hospital Care for Children: What are appropriate methods and urine collection in UTI? J Trop Pediatr 2007;53:221-4.
20. Kubik MJ, McCarter YS. Controversies in the diagnosis of urinary tract infections. Clin Micr Newsletter 2012;34:185-9.